Eur J Vasc Endovasc Surg 11, 414–416 (1996)

Histological Verification of Computerised Carotid Plaque Characterisation

N. M. El-Barghouty*[1], T. Levine[2], S. Ladva[2], A. Flanagan[2] and A. Nicolaides[1]

[1]*Academic Surgical Unit and* [2]*Histopathology Department, St. Mary's Hospital Medical School, London, U.K.*

The correlation between the computerised measurement of carotid plaque echogenicity on high resolution ultrasound imaging and plaque histology was studied in 52 patients undergoing carotid endarterectomy. Thirteen plaques were from asymptomatic patients, 15 were associated with amaurosis fugax, 10 with transient ischaemic attacks and 14 with stroke.

Longitudinal images of the anterior and posterior component of each plaque were obtained by ATL Ultramark-4 Duplex scanner and were transferred to a computer. Using an image analysis program the median of the overall grey scale content of each plaque component was evaluated and used as a measure of echogenicity.

Following carotid endarterectomy each plaque specimen was divided into anterior and posterior component and then fixed, oriented, sectioned and stained in the longitudinal plane corresponding to the ultrasound image. Plaque histology sections were then examined by computer morphometric analysis and the percentage surface areas of fibrous tissue, lipid deposits and haemorrhage were calculated. This was then correlated with the grey scale median for each plaque component.

Plaques with a high lipid and haemorrhage content as established histologically had a low grey scale median (Spearman correlation $r = -0.351$, $p < 0.05$) and those with a high fibrous content had a high grey scale median ($r = 0.411$, $p < 0.001$).

This study has shown that computerised measurement of carotid plaque echogenicity correlates well with histology and could be used to predict plaque composition, thus identifying high risk plaques with high lipid and haemorrhage content.

Key Words: Carotid plaques; Computerised characterisation; Histology.

Introduction

The ultrasonic image can provide unique information on the composition of atherosclerotic carotid plaques, in particular, the relative content of lipid, fibrous tissue and calcific deposits.[1]

To date, however, the study of carotid plaque morphology on ultrasonography has relied upon visual characterisation based on subjective and qualitative evaluation of the B-mode images. Objective and quantitative grading of plaque echogenicity using computerised measurements provides an operator-independent assessment of plaque echoic structure which could prove more accurate than visual characterisation.

It has been suggested that in advanced carotid stenosis, echolucent atherosclerotic plaques with a high lipid and haemorrhage content are unstable and more likely to embolise than echogenic fibrous plaques.[2] It has also been demonstrated that there is preponderance of echolucent plaques in patients with symptomatic cerebrovascular disease, while echogenic plaques are more common in asymptomatic patients.[3] Similarly echolucent plaques are associated with a higher incidence of cerebral infarction as compared to the more stable echogenic plaques.[4]

The aim of this study was to evaluate the correlation between computerised measurement of carotid plaque echogenicity on high resolution ultrasound and plaque histology.

Patients and Methods

Fifty-two consecutive patients undergoing carotid endarterectomy were prospectively studied. Thirteen plaques was from asymptomatic patients, 15 were associated with amaurosis fugax, 10 with transient ischaemic attacks and 14 with stroke. All patients had more than 50% internal carotid artery stenosis on

*Please address all correspondence to: Naif M. El-Barghouty, Woodhorn Gardens, Wide Open, Tyne and Wear, NE13 6AG, U.K.

Duplex scanning. The mean interval between the last ischaemic event and subsequent operation was 8 weeks with a range from less than one week ($n = 2$) to 4 months ($n = 5$).

All scans were performed with an Ultramark 4 ATL duplex scanner (Advanced Technology Laboratories, Letchworth, U.K.) with a 7.5 MHz high - resolution linear array scan head. Scan and printer settings (power output 50%, dynamic range 47 dB, gain, grey scale, filters and ramp) were preset at machine start - up and were not altered during the examinations. All patients were examined supine with slight head tilt. Anterolateral and posterolateral projections were used to image the plaques longitudinally. All images were recorded on photographic paper and reviewed. Anterior and posterior wall carotid plaques of each patient were imaged at the site of their maximum thickness and evaluated separately. The plaque images on photographic paper were scanned using Epson 8000 scanner (Epson U.K. Ltd., Hemel Hempstead, Hertfordshire, U.K.) and the images were transferred to an Apple Macintosh Quadra 840 AV computer (Apple Computer Ltd. Uxbridge, Middlesex, U.K.). Using Adobe photoshop™ 2.5.1 image analysis program (Adope Systems Incorporation, Mountain View, CA 94039, U.S.A.) each plaque was outlined using the computer mouse and its overall grey scale content (from 0 which denotes black to 255 which denotes white) was analysed for the mean, standard deviation, median and total pixel count. The grey scale median was used as a measure of the overall plaque echogenicity. The higher the grey scale median the more echogenic was the plaque.

Following carotid endarterectomy, each specimen was fixed in 10% formaline for 24 h and then oriented and divided into anterior and posterior wall components composed of longitudinal strips of 3 mm thickness. Each strip was embedded in paraffin, processed and cut into 3 μm sections at the site of maximum plaque thickness.

Sections were stained with Haematoxylin and Eosin to assess the overall plaque morphology and to evaluate deposits of calcium, and cholesterol clefts which indicates lipid deposition, elastic Van Giesen to assess the fibrous component of the plaque, Perls stain to assess the iron content indicating previous haemorrhage and Martius scarlet blue to identify the presence of fibrin which indicates recent haemorrhage.

Each section was reviewed under standard light microscopy and the image transferred directly via an attached camera to an image analysis computer (Seescan plc, Cambridge, U.K.). The areas of fibrous tissue, lipid deposits and haemorrhage were separately identified by the operator and outlined on the screen using the computer mouse. Each outlined area was recorded by the computer and converted by the measuring software into a percentage surface area value. The subsequent results were then correlated to the grey scale median for each plaque.

Results

The mean percentage of fibrous tissue content in symptomatic plaques was 70% and that of the asymptomatic plaques was 74% indicating that asymptomatic plaques tended to have more fibrous tissue content than symptomatic plaques although in this study this difference did not reach statistical significance (Wilcoxon test, $p = 0.46$).

The mean percentage of lipid and haemorrhage content of symptomatic plaques was 32% and that of asymptomatic plaques was 30% indicating that symptomatic plaques tended to have more lipid and haemorrhage content although in this study this difference did not reach statistical significance (Wilcoxon test, $p = 0.9$).

As shown in Figs. 1 and 2, the increase in the fibrous tissue content of the plaque was associated with an increase in the grey scale median (Spearman correlation coefficient = 0.412, $p =$ 0.0003) and the increase of the lipid and haemorrhage content of the plaque was associated with a decrease in the grey scale median (Spearman correlation coefficient = –0.352, $p = 0.0381$).

Discussion

Quantitative analysis of carotid plaque echogenicity

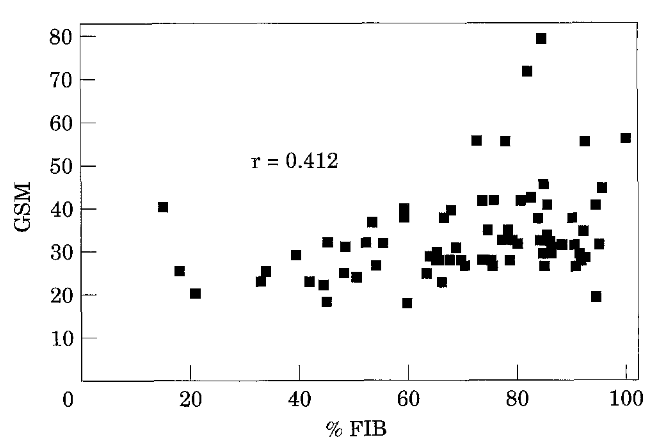

Fig. 1. Showing the correlation between the grey scale median (GSM) and the percentage fibrous tissue content (%FIB) in each plaque.

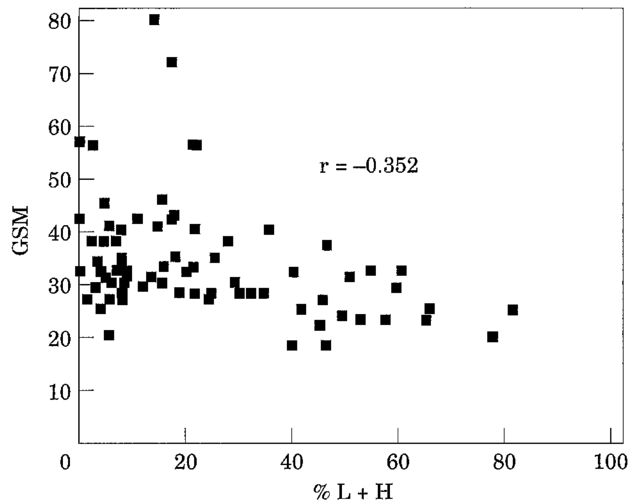

Fig. 2. Showing the relation between the grey scale median (GSM) and the percentage of lipid and haemorrhage (%L + H) in each plaque.

using computerised measurements provides an objective assessment of the plaque ultrasound image and is a more accurate than visual characterisation.

The aim of this study was to verify this computer measurement with histology and to evaluate whether it could predict histological composition of the plaque.

We have shown that plaque computer echogenicity measurement in the form of the grey scale median correlated well with histology. A high fibrous tissue content of the plaque was associated with a high grey scale median and a high lipid and haemorrhage content was associated with a low grey scale median. Thus, it is possible to predict the histological composition of the plaque prior to surgery.

Since it has been suggested that echolucent plaques with a high lipid and haemorrhage content are unstable and more likely to be associated with embolic events than echogenic predominantly fibrous plaques,[2] this method provides a more objective assessment of plaque composition which could be used to identify high and low risk plaques.

This could provide an important stage in the assessment of patients with asymptomatic carotid stenosis as it has been shown that those patients with echolucent carotid plaques have a higher risk of subsequent neurologic events as compared to patients with echogenic plaques.[5]

Evaluation of the different echo zones within the carotid plaques using complex quantitative analysis of the integrated back scatter ultrasound signal was found to be effective in distinguishing lipidic, fibrotic and calcific components within the plaques.[6] Our study however, present a more practical and simpler method for the evaluation of the overall carotid plaque echogenicity which correlates well with plaque histological composition.

Lusby *et al.*,[7] reported that 92% of patients undergoing carotid endarterectomy for symptomatic cerebral ischaemia had evidence of intraplaque haemorrhage in their operative specimens compared to 27% of specimens from asymptomatic patients. The mean interval between the onset of symptoms and operation was 2.9 weeks. In contrast, Feeley *et al.*[8] identified intraplaque haemorrhage in 33% of carotid endarterectomy specimens with no significant difference between symptomatic and asymptomatic plaques. In this study, however, the mean interval between presenting symptoms and subsequent surgery was 16 weeks, which would probably allow for healing and remodelling following haemorrhage. In addition, they demonstrated signfiicantly greater fibrous content with corresponding less lipid material in asymptomatic as compared to symptomatic plaques.

In this study, we have shown that asymptomatic plaques tended to have more fibrous tissue and less lipid and haemorrhage content than symptomatic plaques although this did not reach statistical significance. This may reflect insufficient number of cases studied in each group or due to the length of the interval between the last ischaemic events and operation (8 weeks).

References

1 Reilly L, Lusby R, Hughes L *et al.* Carotid plaque histology using real-time ultrasonography. *Am J Surg* 1983; **146**: 188.
2 Bock R, Gray-Weale A, Mock P *et al.* The natural history of asymptomatic carotid artery disease. *J Vasc Surg* 1993; **17**: 160–171.
3 Geroulakos G, Ramaswami G, Nicolaides A *et al.* Characterisation of symptomatic and asymptomatic carotid plaques using high-resolution real - time ultrasonography. *Br J Surg* 1993; **80**: 1274–1277.
4 Geroulakos G, Domjan J, Nicolaides A *et al.* Ultrasonic carotid artery plaque structure and the risk of cerebral infarction on computed tomography. *J Vasc Surg* 1994; **20**: 263–266.
5 O'Holleran L, Kennelly M, McClurken M, Johnson J. Natural history of asymptomatic carotid plaque. *Am J Surg* 1987; **154**: 659.
6 Urbani M, Picano E, Parenti G *et al.* In vivo radio frequency-based ultrasonic tissue characterisation of the atherosclerotic plaque. *Stroke* 1993; **24**: 1507–1512.
7 Lusby R, Ferrell L, Ehrenfeld W *et al.* Carotid plaque haemorrhage : Its role in production of cerebral ischaemia. *Arch Surg* 1982; **114**: 1479.
8 Feeley T, Leen E, Colgan M *et al.* Histologic characteristics of carotid artery plaque. *J Vasc Surg* 1991; **13**: 719–724.

Accepted 27 January 1995